

EFFECTS OF THE FOLIAR SPRAY OF GROWTH REGULATORS ON THE FATTY ACID COMPOSITION OF SAFFLOWER UNDER ORGANIC AND CHEMICAL SOIL FERTILIZATION

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Abstract: In the current study, we evaluated the impacts of organic and chemical fertilizers as well as the foliar application of growth regulators on quantitative traits and seed oil content of safflower grown in northwestern Iran. The experiment was done as a split-plot (3×5), and the main plot was assigned to different fertilizers, including organic fertilizer (FYM: farmyard manure 20 t ha^{-1}), full chemical fertilizer (FCF) and no fertilization “control” (NF). The sub-plots were allocated to foliar spray treatments, which included control (no-spray application; S_0), choline (S_1), chitosan (S_2) and salicylic acid (S_3). The results showed that the application of fertilizers significantly affected the oil and protein content of the seed. However, the effect of FYM was more prominent than FCF on oil qualitative characteristics. A significant positive correlation was observed between oil content, protein content, and some fatty acid composition such as oleic acid, arachidonic acid, stearic acid, and palmitic acid. The highest values of the previously mentioned traits were obtained using $FYMS_1$, $FCFS_1$ and $FYMS_3$. This trend was also clearly obvious in the content of linoleic acid as the main fatty acid in safflower oil. Among the foliar spraying treatments, the most improving effect was obtained with the use of choline. In conclusion, improving soil conditions through the application of FYM and appropriate amounts of chemical fertilizers is one of the most important agronomic management measures to improve the oil quality of safflower seeds.

Key words: choline, fatty acid composition, linoleic acid, iodine value, quantitative analysis.

Introduction

Safflower (*Carthamus tinctorius* L.) is an annual, broadleaf oilseed crop and a member of the family Compositae or Asteraceae, but also it can be considered a

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multi-purpose plant. The safflower plant provides a useful vegetable and cosmetic oil and attractive dyes for textiles. It is also used for extracting oil from seeds that are used as edible oil or in the paint industry. Today, this crop supplies oil, meal, birdseed, and some by-products (residue from oil processing) for the food and industrial product markets, although this crop is now primarily grown for the oil (Zemour et al., 2021). It originates from the Asian Fertile Crescent, and previous evaluations clearly show that safflower has a good ability to adapt to drought and high temperatures (Zemour et al., 2019; Yeloojeh and Saeidi, 2020). Therefore, its introduction in semi-arid regions would constitute an alternative to the development of oilseed crops.

However, under semi-arid conditions, soil fertility in systems with low and asymmetric annual precipitation, hot summer and cold winter, is constrained by environmental extremes. In most areas with a semi-arid climate, these soils have inherently low fertility, low availability of nitrogen and phosphorus, low water-holding capacity, high pH, low soil organic matter (ranging from 0.1 to 3%), shallowness, stoniness, and other specific problems (Garcia-Franco et al., 2018). However, climate change during the last decades has intensified these restrictions. Semi-arid areas in the West Asia and North Africa (WANA) are vast and significant. Hence, these lands are of great global significance even if their agricultural production potential is relatively low. However, proper soil fertility management is of prime importance in increasing crop production. In this moor, it seems that the improvement of soil conditions and nutritional statuses of the plant and increasing vegetative growth also affect the qualitative components of the extracted oil.

In the above conditions, it appears that incorporating organic matter can have several benefits for agricultural soils. Organic matter causes soil to clump and form soil aggregates, improving soil structure. With better soil structure, permeability (infiltration of water through the soil) improves, in turn improving the soil's ability to take up and hold water (Barton et al., 2016). Farmyard manure is a key fertilizer in organic and sustainable soil management. The successive application of farmyard manure can also increase crop production and has the potential to reduce chemical fertilizer requirements. Because of the development of the livestock industry, farmyard manure is an available, relatively cheap and potential source of multiple nutrients for improving soil chemical, physical and biological properties (Nouraein et al., 2019).

In addition, it appears that some foliar spraying treatments can provide better plant growth in semi-arid conditions by stimulating defense mechanisms and growth encouragement (Gajc-Wolska et al., 2018). Chitosan (Ch) is a carbohydrate derivative from chitin, and it seems that its foliar application enhances the protection of crops against fungal attacks and reduces disease severity (El Amerany et al., 2020). Choline is a water-soluble vitamin recognized as a growth regulator under multiple stress conditions by improving growth, oxidative defense, and

secondary metabolism (Riaz et al., 2021). Also, salicylic acid (SA) is a plant hormone that has been described to play an essential role in the activation and regulation of multiple responses to biotic and abiotic stresses, and it has been revealed that spraying plants with SA solution is favorable for plant growth and helps to protect them against abiotic stresses (Maruri-López et al., 2019).

Although some studies have previously examined the effects of foliar application, a number of questions regarding differences in their impact under different nutritional conditions remain to be addressed. The major objective of this study was to investigate the effect of the foliar application of growth regulators under chemical and organic fertilizer management on safflower oil quality.

Material and Methods

Site description

The response of safflower fertilizer management and foliar spray of growth regulators were studied at the Research Farm in the Kharajou region, Northwestern Iran (longitude: 46°53'E; latitude: 37°31'N; altitude: 1780 m) during the growing season of 2016–2017 under irrigated conditions. The study area is a part of regional semi-arid highlands; in terms of climate, the area has been classified (according to the Köppen and Geiger classification system) as a cold semi-arid area (Peel et al., 2007). The annual average precipitation is 310 mm, with a maximum in April, and one-third of the precipitation includes snow, and about 78% of the precipitation occurs during the growing seasons. The previously fallowed field was prepared by plowing three times with a tractor-mounted cultivator and planking with the last plowing. The site was covered by a fine mixed, mesic, typical calcixerpts soil, exhibiting a xeric moisture regime. The soil was clay loam texture and contained 2.34 g.kg⁻¹ organic matter, 0.54 g.kg⁻¹ total N, 9.38 mg.kg⁻¹ available phosphorus (P), and 171.28 mg.kg⁻¹ available potassium (K) in the 0–20-cm soil layer. The organic matter content of the soil was low (<0.5%) at the initiation of the experiment. Decomposed farmyard manure was applied annually, and tillage operations were performed in early March.

Experimental design

Seeds of the hardy facultative safflower (*Carthamus tinctorius* L.) CV. 'Esfahan' were purchased from the Pakan Bazr Company. The experiment was laid out according to a split-plot (3×4), with main plots arranged as an RCBD with three replications and a net plot size of 5 × 4 m. The main plot was assigned to different fertilizers, including organic fertilizer (application of 20 t ha⁻¹ farmyard manure), full chemical fertilizer and unfertilized condition "control" (C). The sub-plots were allocated to foliar spray treatments, which included control (no-spray application), choline, chitosan and salicylic acid (100 ppm). Full chemical fertilizer included 200 kg ha⁻¹ N-P-K (20:10:5) fertilizer and 10 kg ha⁻¹ complete

micronutrient fertilizer (Fe = 7%, Zn = 3.7%, Mn = 3%, Cu = 0.25%, B = 0.25%, Mo = 0.35%) which were used as two split applications, i.e. a half as a pre-plant (starter fertilizer) and a half as a post-emergence side-dress application during stem elongation. Each plot included twenty rows, 5 m long and 75 cm apart. Seeds were sown 20 cm apart at 5-cm depth. In the interspaces, the small terraces of 1.5 m were considered to prevent contamination by surface run-off containing fertilizers. Irrigation was applied every week to replace soil water lost due to evapotranspiration. Growth regulators were sprayed over the foliage to the point of run-off during the stem elongation (BBCH=30), flowering (BBCH=30) and head growth (BBCH=71). Low weed, disease, insect and pest pressures were maintained by using cultivation and recommended amounts of pesticides and herbicides.

The evaluation of fatty acids

The harvest occurred 140 days after the emergence of the plants. The plants were collected from 1 m² of each plot before threshing, and manual cleaning of the seeds was performed. Total protein was estimated by the Kjeldahl method according to Nosheen et al. (2016). The moisture content was determined using the gravimetric method by drying a sub-sample for 24 h at 105°C. To evaluate the oil content, the extraction was performed using a Soxhlet extractor under laboratory conditions using petroleum ether solvent. The extraction was performed with 2 g of ground seeds. The iodine value of oil was determined according to the standard method (Cd 1-25) introduced by AOCS (1993). The saponification value was measured according to the standard method (Cd 3-25) suggested by AOCS (1993). The fatty acid compositions were analyzed according to Pasandi et al. (2018) by GC-MS and GC-FID instruments. The determination of the fatty acid profile was carried out by GC equipped with a flame ionization detector (Agilent 6890N, USA) and a TC-FFAP capillary column (60 m×0.25 mm internal diameter, 0.25 µm). The temperature program was as follows: starting at 150 °C and then heating to 190 °C at 5 °C/min, after 2 min followed by heating from 190 °C to 250 °C at 5 °C/min. The final temperature (250 °C) was held for 8 min. The fatty acids used as standards for the GC analyses (palmitic, stearic, and oleic acids) were from Sigma. The injector and detector temperatures were both set at 250 °C. Injections of the methylated sample (1 µL) were made in the splitless mode. To convert the fatty acid compositions to fatty acid methyl esters, 0.2 mL of 2 N KOH and 1.5 mL of hexane were added to 0.1g of safflower oil. The mixture was then centrifuged at 2500 rpm for 1 min. Then, the FAME was separated and analyzed.

Statistical analysis

Data analysis was conducted using SAS version 9.4 (SAS Institute Inc). Mean values were compared according to Steel and Torrie (1980) by least significant difference (LSD) at $P < 0.05$.

Results and Discussion

The highest seed protein contents were recorded for plants grown under FYM applied conditions, which were followed by the FCF. The foliar application of choline had the greatest effect on increasing the seed protein content, followed by the foliar application of chitosan and salicylic acid. Interestingly, choline foliar application was able to increase the protein content even under NF conditions (Figure 1).

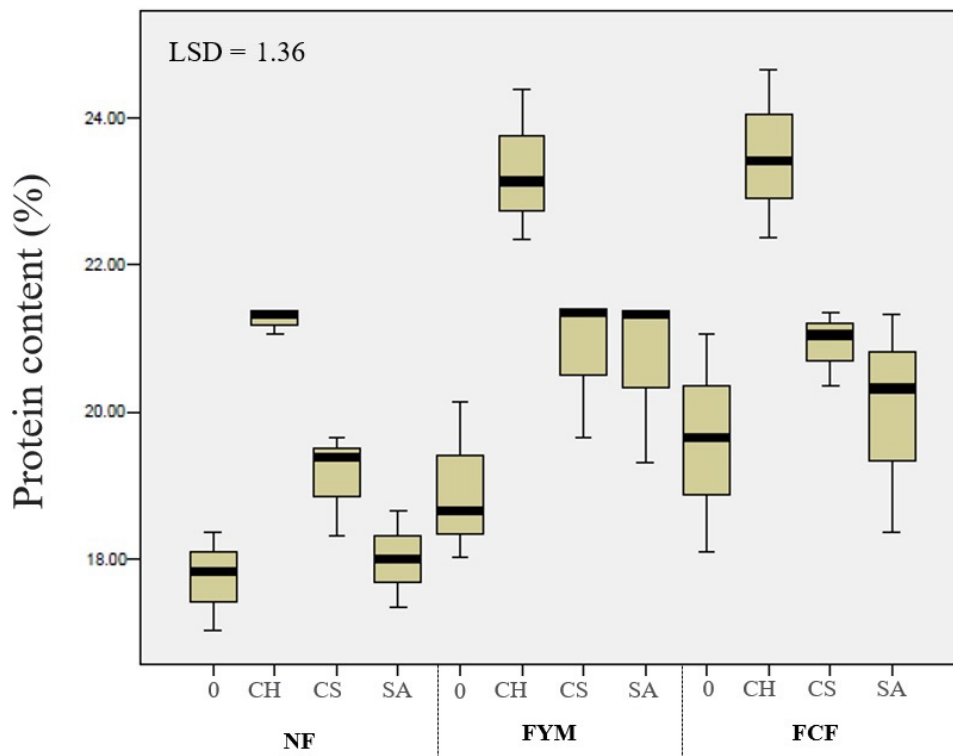


Figure 1. Effects of fertilizer application and foliar spray treatments on the seed protein content of safflower plants grown in a semi-arid region in northwestern Iran. NF: no fertilizer application, FYM: farmyard manure, FCF: full chemical fertilizer, S₀: control (no-spray application), S₁: a foliar spray of choline, S₂: a foliar spray of chitosan, S₃: a foliar spray of salicylic acid. The cap bar refers to the standard error. The dark horizontal line in each box indicates the average combined treatment. The columns with a difference higher than LSD (least significant difference) are statistically different at the $P < 0.05$ level.

The results of the analysis of variance (ANOVA) showed that seed oil and protein content were significantly affected by fertilizers and foliar sprays ($p <$

0.01). The highest oil content was recorded in plants grown under FYM or FCF applied conditions and sprayed with choline. Although all foliar treatments improved the oil content when compared with control (no-sprayed plants), the salicylic acid had a slightly greater improving effect than chitosan. The positive effect of the choline spray on seed oil content was quite evident regardless of fertilizer conditions (Figure 2).

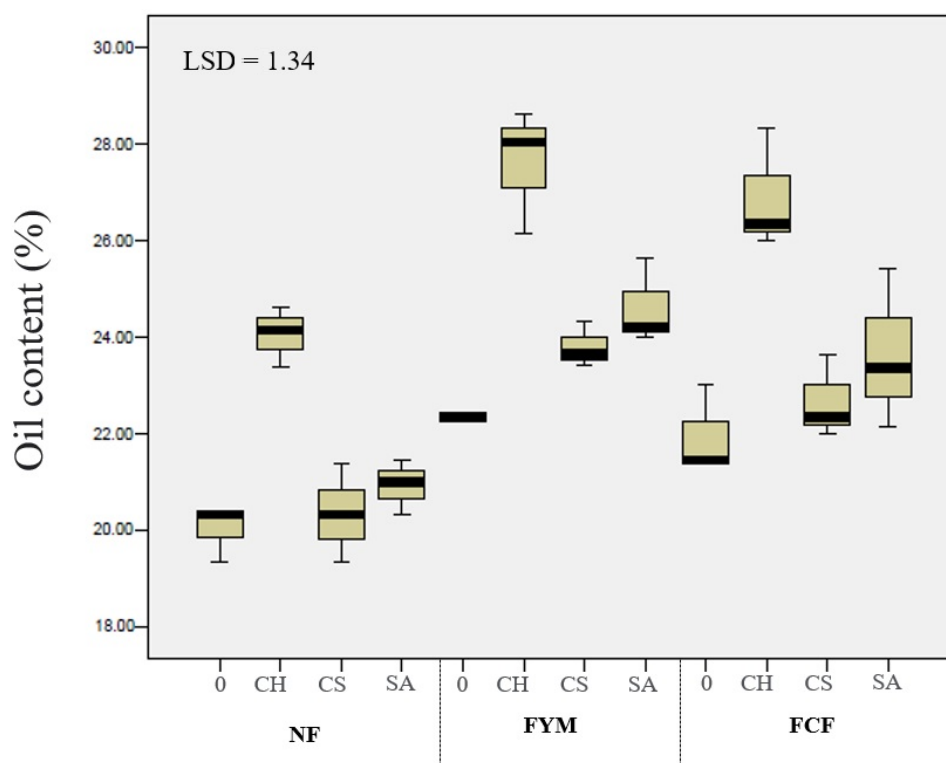


Figure 2. Effects of fertilizer application and foliar spray treatments on extracted oil content from safflower plants grown in a semi-arid region in northwestern Iran. NF: no fertilizer application, FYM: farmyard manure, FCF: full chemical fertilizer, S₀: control (no-spray application), S₁: a foliar spray of choline, S₂: a foliar spray of chitosan, S₃: a foliar spray of salicylic acid.

Table 1 lists the most abundant fatty acids found in the analyzed oil as affected by soil fertilization and foliar sprays. The oil contents of safflower seeds ranged from 20.08% to 29.51%. Oil analysis revealed that the main fatty acid seed samples were linoleic acid (67.51% ± 1.26%), oleic acid (6.30% ± 0.52%), palmitic acid (5.83% ± 0.7%), stearic acid (2.98% ± 0.35%), arachidonic acid (1.01% ±

0.04%), linolenic acid ($2.31\% \pm 0.2\%$), lauric acid ($1.63\% \pm 0.17\%$) and myristic acid ($1.48\% \pm 0.14\%$). The evaluation of seed oil content showed that the main effect of fertilizers and foliar sprays was statistically significant regarding this seed quality parameter ($p < 0.05$). ANOVA showed that fertilizers and foliar sprays significantly affected the linoleic acid content. FYM and FCF increased linoleic acid content by 10% and 8% compared to control. Also, the choline spray on plants increased linoleic acid content by 9.3%.

Table 1. Effects of foliar sprays of growth regulators under organic and chemical soil fertilization on the percentage of fatty acids in safflower seeds.

Treatment	PA	SA	OA	LA	LL	AC	LU	MA
Control	4.41±0.45	2.45±0.20	4.40±0.46	62.98±1.32	1.04±0.01	0.68±0.02	1.43±0.21	1.62±0.09
NF	Choline	5.74±0.47	2.49±0.43	6.63±1.41	66.80±1.27	1.27±0.11	0.80±0.09	2.06±0.05
	Chitosan	4.45±0.53	2.64±0.09	5.15±0.70	63.57±0.87	1.06±0.15	0.83±0.10	2.62±0.04
	Salicylic acid	3.66±0.33	2.22±0.07	4.46±0.31	62.76±1.44	1.22±0.09	0.69±0.03	2.01±0.19
FYM	Control	5.21±0.50	3.02±0.08	6.49±0.64	68.32±1.65	3.01±0.46	1.08±0.00	1.20±0.09
	Choline	8.40±0.13	3.96±0.62	9.25±0.04	73.68±0.67	4.77±0.29	1.40±0.02	1.40±0.12
	Chitosan	6.01±0.88	2.96±0.42	7.27±0.19	68.67±1.28	3.31±0.57	1.12±0.04	1.39±0.33
	Salicylic acid	6.85±0.89	3.43±0.40	6.65±0.63	68.26±1.29	3.54±0.25	1.29±0.01	1.57±0.18
FCF	Control	6.25±1.16	3.42±0.74	5.23±0.49	66.04±1.26	1.98±0.11	0.91±0.04	1.16±0.09
	Choline	7.81±1.30	3.46±0.40	7.32±1.00	72.97±1.22	2.36±0.12	1.12±0.00	2.11±0.23
	Chitosan	5.76±1.10	3.06±0.56	6.17±0.06	68.00±1.24	2.15±0.10	1.08±0.11	1.55±0.47
	Salicylic acid	5.42±0.68	2.69±0.24	6.64±0.40	68.11±1.71	2.04±0.20	1.02±0.07	1.18±0.07
LSD	1.80	1.02	1.44	3.22	0.60	0.13	0.52	0.46

NF: no fertilizer application, FYM: farmyard manure, FCF: full chemical fertilizer, PA: palmitic acid, SA: stearic acid, OA: oleic acid, LA: linoleic acid, LL: linolenic acid, AC: arachidonic acid, LU: lauric acid, MA: myristic acid. In each column, if the difference between the means is greater than the LSD (Least Significant Difference) value, then the means are significantly different ($P \leq 0.05$).

The mean comparison of palmitic acid content among different foliar spraying treatments also showed the beneficial effects of choline foliar application on improving the amount of this fatty acid. However, the effect of other foliar spraying treatments was different under organic and chemical fertilizer conditions, so the greatest effect of foliar spraying treatments was observed under FYM applied conditions. Furthermore, the foliar application of salicylic acid under NF or FCF conditions caused a significant reduction in the content of this fatty acid. The examination of oleic acid content also showed the effectiveness of choline foliar application so that oleic acid content in plants sprayed with choline was 71%

higher than in no-sprayed plants, while the highest amount of oleic acid was observed in plants grown under FYM applied conditions (Table 1). The evaluation of linolenic acid and arachidonic acid content revealed that the effect of foliar spraying treatments, especially choline, was only visible and significant under FYM and FCF applied conditions.

Responses of lauric acid content to fertilizer treatments were somewhat different from other fatty acids, so the highest amount of this fatty acid was observed under no fertilization conditions. The highest amount of lauric acid (2.06%) was obtained by using chitosan under NF conditions. The mean comparison of myristic acid amount between different treatments surprisingly showed that the application of chemical fertilizers reduced the amount of this fatty acid by 173% compared to the NF conditions (Table 1). Our results showed that fertilizer application significantly affected oil qualitative characteristics. It is important to highlight the fact that soils of semi-arid regions with the Mediterranean climate are faced with serious limitations in terms of nutrient supply for the plant and physical and chemical properties due to low precipitation and lack of proper crop management. Since the experiment was performed under full irrigation conditions, it appears that the application of organic and chemical fertilizers by improving the soil physicochemical properties and providing the essential elements has provided better plant growth and a sufficient supply of photoassimilates. Under such conditions, improving the source-sink relationship in the plant leads to a greater supply of amino acids needed for protein biosynthesis in the filling seeds, as well as the precursors needed for fatty acid biosynthesis and increases the seed oil content (Poisson et al., 2019). The protein and oil contents of seeds are crucial factors for farmers and industry. However, up to the present time, the impact of soil fertilization and foliar sprays on the oil quality of the seed has rarely been taken into account in safflower, which could be re-introduced as a forgotten and drought-tolerant oil crop in semi-arid regions.

The quality of vegetable oil is highly related to fatty acid composition and the ratio of saturated to unsaturated fatty acids. The increasing amount of unsaturated fatty acids increases the quality, however, it will result in a decrease in the shelf life of the produced oil. Although the application of fertilizers and foliar sprays increased almost all saturated and unsaturated fatty acids, the highest numerical increase was related to unsaturated fatty acids (oleic acid; 18:1 and linoleic acid; 18:2). The increasing unsaturated fatty acid content with soil fertilization could be attributed to the improvement of nutrient availability and supply for the oil metabolism (Pasandi et al., 2018). This result ties well with previous studies wherein the inoculation of arbuscular mycorrhizal fungus on the soybean seed improved the oil quality and unsaturated fatty acid content (Amani Machiani et al., 2021).

The results of correlation analysis were corroborated by principal component analysis (PCA) (Figure 3). The correlation coefficient between any two traits is approximated by the cosine of the angle between their vectors. In Figure 3, the most prominent relation was the strong positive association between protein content, palmitic acid, stearic acid, oleic acid, linoleic acid, linolenic acid, arachidonic acid content and iodine value. Furthermore, PCA analysis showed that the highest values of mentioned traits were obtained by the foliar spray of choline under FYM and FCF conditions (FYMS₁ and FCFS₁). The increasing worldwide demand for vegetable protein for human nutrition (vegetarian or vegan diets) has led to a wider search for sources of vegetable protein, thus making safflower seed proteins interesting alternatives due to their acceptable quality. In our study, a strong linear positive correlation between protein content and oil content was determined. This might be due to increased source strength for photoassimilates supply and increased photoassimilate utilization (sink strength). These findings are directly in line with previous findings (Janmohammadi et al., 2014; Nouraein et al., 2019).

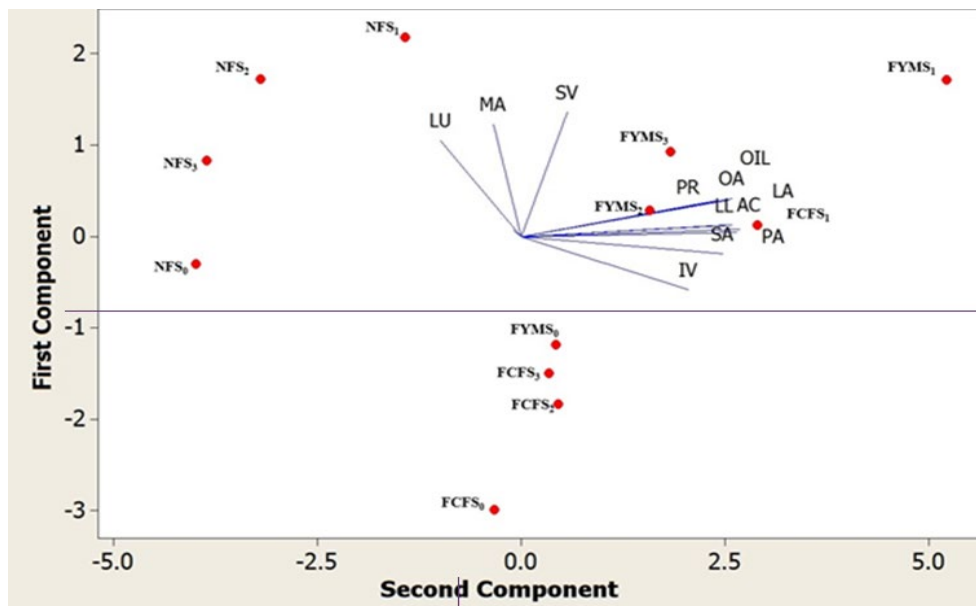


Figure 3. The bi-plot of the first two principal components (PC1 and PC2) for the spatial distribution of seed oil qualitative traits and combined treatment (fertilizer \times foliar spray). NF: no fertilizer application, FYM: farmyard manure, FCF: full chemical fertilizer, S₀: control (no-spray application), S₁: a foliar spray of choline, S₂: a foliar spray of chitosan, S₃: a foliar spray of salicylic acid, PA: palmitic acid, SA: stearic acid, OA: oleic acid, LA: linoleic acid, LL: linolenic acid, AC: arachidonic acid, LU: lauric acid, MA: myristic acid, IV: iodine value, SV: saponification value.

The assessment of the iodine value of oil showed that fertilizer application significantly affected this parameter. The mean comparison of the iodine value between combined treatments showed that the application of organic and chemical fertilizers increased the iodine value (Figure 4).

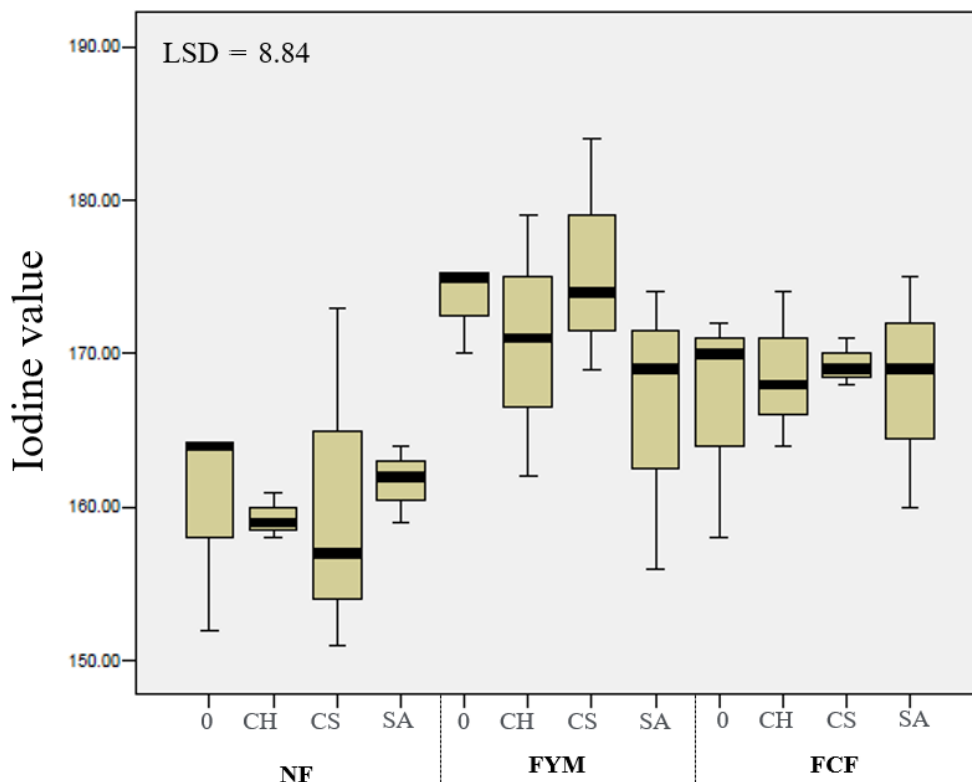


Figure 4. Effects of fertilizer application and foliar spray treatments on the iodine value of the safflower seed oil extracted from plants grown in a semi-arid region in northwestern Iran. NF: no fertilizer application, FYM: farmyard manure, FCF: full chemical fertilizer, S₀: control (no-spray application), S₁: a foliar spray of choline, S₂: a foliar spray of chitosan, S₃: a foliar spray of salicylic acid.

The evaluation of the iodine index indicates that with improving the plant growth conditions, the number of unsaturated fatty acids in the oil increased. In agreement with our hypothesis, a noticeable change in oil characteristics was recorded in response to the soil fertilization management and foliar sprays. Superior results are seen for distinguished effects of choline spray compared to other foliar treatments. Choline is an organic nitrogen compound, and it is the precursor of the osmoprotectant glycine betaine, and also it protects cells against

oxidative damage by reactive oxygen species (Salinas et al., 2013). Under certain assumptions, its distinctive effects can be attributed to its properly utilized concentration, its chemical compatibility with the nature of cell membranes and its greater penetration, stimulation of defense processes and improvement of plant growth. Our results highlighted synergetic effects on soil fertilization (especially with FYM) and the foliar use of growth regulator or protector efficiencies to improve the oil quality of safflower.

Our results showed that the efficiency and effectiveness of foliar spraying treatments were only evident and significant when the plant was grown under adequate nutritional status and suitable soil conditions. Based on these results, it is clear that soil management is a higher priority in the studied area. Our results cast a new light on the application of FYM as an inexpensive, accessible and eco-friendly source to improve seed oil quality. A difference between the effects of FYM and FCF can only be attributable to the positive and significant effects of FYM on improving soil physical properties. It is worth discussing these interesting facts revealed by the results of Nouraein et al. (2019). Overall, the promising finding was that improving the physical and chemical conditions of the soil and providing an essential situation for basic plant growth can significantly increase the effectiveness of foliar treatments and improve seed oil quality.

Conclusion

In conclusion, this study showed that the different fertilization or foliar spray treatments resulted in different responses of qualitative oil composition. Although the application of organic and chemical fertilizers increased the oil and protein contents compared to the control (no fertilization), the improving effect of FYM application on the quality aspects of oil was greater than that of chemical fertilization. Among the foliar spraying treatments, the greatest effect was related to choline, so even under no fertilization conditions, some of its positive effects were observed. Our results showed that with the improvement of plant nutritional conditions through the application of fertilizers, the effect of foliar application treatments on oil properties became more noticeable. In summary, the application of the organic fertilizer with the foliar spray of choline had the greatest impact on the composition of safflower seed oil and improved oil quality. Future studies could fruitfully explore this issue further by focusing on biochemical pathways of oil biosynthesis in seeds during filling stages under different nutritional statuses of the plant.

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I ORGANSKIH I MINERALNIH ĐUBRIVA NA
SASTAV MASNIH KISELINA ŠAFRANIKE

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R e z i m e

U ovoj studiji procenjivali smo uticaje organskih i mineralnih đubriva, kao i folijarnu primenu regulatora rasta na kvantitativne osobine i sadržaj ulja u semenu šafranke koja se uzgaja u severozapadnom Iranu. Eksperiment je postavljen kao podeljena parcela (3×5), a glavna parcela je imala tretmane različitim đubrivima, uključujući organsko đubrivo (ST: stajnjak 20 t ha^{-1}), mineralno đubrivo (MĐ) i bez đubrenja – 'kontrola' (BĐ). Na potparcelama su bili folijarni tretmani regulatorima rasta, koji su uključivali kontrolu (bez tretmana; S_0), holin (S_1), hitozan (S_2) i salicilnu kiselinu (S_3). Rezultati su pokazali da primena đubriva značajno utiče na sadržaj ulja i proteina u semenu. Međutim, uticaj stajnjaka je bio izraženiji od uticaja mineralnog đubriva na kvalitativne karakteristike ulja. Uočena je značajna pozitivna korelacija između sadržaja ulja, sadržaja proteina i sastava nekih masnih kiselina kao što su oleinska kiselina, arahidonska kiselina, stearinska kiselina i palmitinska kiselina. Najveće vrednosti prethodno navedenih osobina dobijene su korišćenjem STS_1 , $MĐS_1$ i STS_3 . Ovaj trend je takođe bio očigledan u sadržaju linolne kiseline kao glavne masne kiseline u ulju šafranke. Među folijarnim tretmanima, najviše poboljšanja postignuto je upotrebom holina. Poboljšanje osobina zemljišta primenom stajnjaka i odgovarajućih količina mineralnih đubriva je jedna od najvažnijih agrotehničkih mera za poboljšanje kvaliteta ulja semena šafranke.

Ključne reči: holin, sastav masnih kiselina, linolna kiselina, količina joda, kvantitativna analiza.

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